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A geographical study on *Pseudaulacaspis pentagona* and its parasitoids in Hungarian highway margins using pheromone traps and molecular markers

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ABSTRACT

A study has been conducted to monitor geographical spread of the white peach scale *Pseudaulacaspis pentagona* (Targioni Tozzetti) (Hemiptera: Diaspididae) (WPS) and its parasitoid populations in 32 stops of the Hungarian highways (M0. M1, M3, M5 and M7) using pheromone traps during 2009 and 2010. In addition to the data collected in the current study, previous data were used to investigate the population trend of this pest from 2007 to 2010. The number of males recorded in traps placed on highways was much lower than in the sites close to urban areas (M0). Our data support results of previous studies which suggest the spreading of white peach scale by vehicles ("transport vector"). The significant decrease in the WPS male catches from 2007 to 2010 might indicate the lowering of the population levels of this pest in the area of the study. Eight hymenopterous parasitoid species were captured in pheromone traps. *Coccophagus* sp. was the predominate species in pheromone traps of WPS in M7, however they may be associated with another coccid species. The identity of scale males and some parasitoids was proved by molecular markers.

KEY WORDS: *Coccophagus* sp., *Encarsia berlesei,* host finding, kairomone, *Signiphora* sp., *Thomsonisca amathus,* vehicles.

Introduction

The white peach scale, *Pseudaulacaspis pentagona* (Targioni–Tozzetti, 1880) is one of the most important pests of ornamentals and fruit trees (Kosztarab and Kozár 1988, Miller and Davidson 2005). This species shows a substantial northward spread in different parts of Europe in the last years (Kozár et al. 1997, Ben-Dov et al. 2011). The most sensitive method for monitoring its spread is the pheromone trap.

Scale insects are often inconspicuous and their detection at sub-economic levels can be difficult. Pheromones represent one of the major components of ecologically based insect pest management (Howse et al. 1998). The pheromone traps provide an efficient tool to study population dynamics (Cravedi and Mazzoni 1993, Kozár and Sheble 1996) but also for mass trapping, mating disruption, or lure-and-kill control strategies (*e.g.*, Smetnik et al. 1991, Kozár 2009).

The use of pheromone traps to monitor scale insect populations requires some basic knowledge of the flight behavior of males

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because of males live for a very short time, about 14 hours. The duration of activity of males is even shorter (Moreno et al. 1974) and it is already substantially reduced after 7 hours.

Sheble and Kozár (1995) found two generations for *P. pentagona* in Hungary. The beginning of the first generation (usually in the first week of June) did not show considerable variation from that in Italy and southern France (Kosztarab and Kozár 1988, Cravedi and Mazzoni 1993, Kozár et al. 1997). On the other hand, the beginning of the second flight period varied from mid-July to mid-August in France (Cravedi and Mazzoni 1993).

Parasitoids often use chemical stimuli, called infochemicals (Franco et al. 2008) when they are searching for their hosts, and these infochemicals can be associated to their host, such in the case with the host sex pheromone. Parasitoids can orientate towards these cues over a moderate distance (Godfray 1994). However, this aspect has not been studied in detail to date in scale insects.

The highway is a relatively new ecological habitat which offers the bases of "road ecology" (Talley 2007, Talley and Holyoak 2009). It forms a connecting ecological corridor for different living organisms across Europe (Kozár et al. 2010). On a large geographical scale, a reliable basis to record the spread of insects is offered by distribution maps, such as the map series published by the Commonwealth Institute of Entomology or those of Kozár and Konstantinova (1981b). It was established, for example, that between 1971 and 1976 the frequency of San Jose scale, *Diaspidiotus perniciosus* (Comstock, 1881) (SJS) decreased and that of *Epidiaspis leperii* (Signoret 1869) increased in Hungary.

The objective of this work was to: (i) monitor the seasonal quantitative changes of WPS male populations in the main Hungarian highways in comparison with the previous data of 2007 and 2008 (Kozár 2009) (ii) collect evidence on the geographical distribution of WPS parasitoids. Collectively, these data will allow us a better understanding of the geographical spreading and distribution of this pest and its parasitoids.

Materials and Methods

During the period 2009-2010 we studied the flight periods of male *P. pentagona* and its parasitoids in five main Hungarian highway margins (M0, M1, M3, M5, and M7) using pheromone traps. The sites (stops) where the pheromone traps were placed at each highway are presented in Table 1 and Figure 1. The distance between two successive stops on each highway ranged between 40 and 50 km. Hungarian tent trap design (10×10 cm, plastic square with plastic glue) was used. Commercial pheromone dispensers of WPS were obtained from Biochemtech (Biochemtech Ltd. Kishinev, Moldavia) pheromone dispensers, with Soveurode (Witasek Pflanzenschutz GmbH, Austria) glue. The compound of the sex pheromone of WPS is (*Z*)-3,9-dimethy-6-isopropeny1-3,9-decadien-1-yl propanoate (WPS-1) (Heath et al. 1980).

The pheromone dispenser was suspended ca 3-4 cm below the trap upper margin. The dispensers were replaced by new ones in ca 4-8 weeks, according to Rice et al. (1982). The traps were placed on *Prunus* sp., *Malus pumila* Mill., and *Syringa vulgaris* L. trees which are common host plants of the WPS (Ben-Dov et al. 2011).

The traps were used during the period of first and second generation of *P. pentagona* throughout 2009 and 2010. The number of traps used in each stop site of the highways is given in Table 1. The trap squares were collected and examined under stereo binocular microscope to record the number of attracted WPS males and its parasitoids.

TABLE 1. The different stops and the respective number of traps placed in 2009 and 2010 at the Hungarian highways during 2007-2010.

M0	M1	M3	M5	M7
Annahegyi p.B. (1,1)	Óbarok p. B. (2,1)	Szilas p. B. (2,2)	Inárcsi p. B. (2,1)	Budaörs (Tesco) p. B. (2,2)
Csepeli p. B. (2,1)	Turul p. B. (1,1)	Kisbag p.B. (1,1)	Lajosmizse p. B. (1,1)	Velence p. B. (2,1)
Alacska p, B. (1,1)	Bábolna p. B. (2,1)	Ecséd p. B. (2,1)	Kecskeméti p. B. (2,1)	Töreki p. B. (2,2)
Vecsés p. B. (1,1)	Arrabona p. B. (1,1)	Gelej p. B. (1,1)	Szatymazi p. B. (1,1)	Táska p. B. (1,1)
Ferihegy p. B. (2,2)	Moson p. B. (2,1)	Polgár p. B. (1,1)	Röszke p. B. (2,1)	Letenye p. B. (2,3)
Dunakeszi p. B. (1,1)		Nyiregyházi p. B. (1,1)		Murakerest p. B. (1,1)
		Záhony p. B. (2,1)		Pince p. B. Slovenia (1,1)
		Debrecen p. B. (1,1)		Güttlesbrun p. B. Austria (1,1)

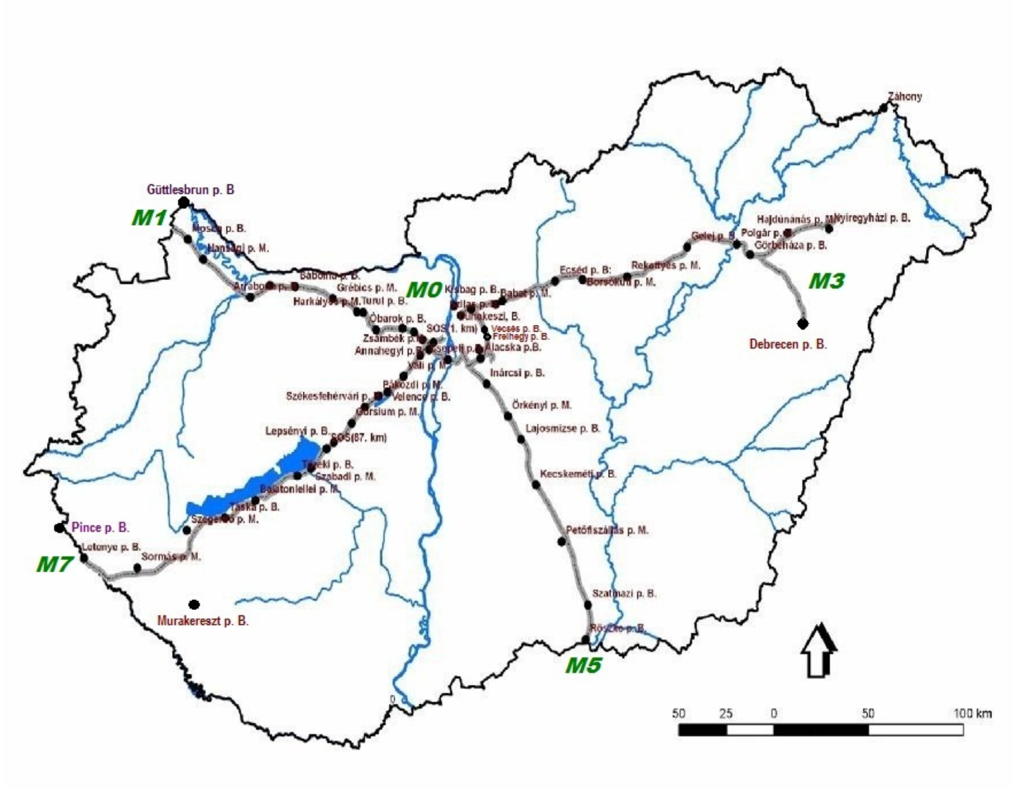

FIG. 1. The different inspected stops of the five main Hungarian highways (M0, M1, M3, M5, and M7) during the period of 2007-2010.

The identity of *P. pentagona* males and *T. amathus* parasitoid was confirmed by molecular analysis (RAPD-PCR) (Frey and Frey 1995). The amplified ITS2 sequences were compared with the similar sequences in the Genebank to ensure the correct identification. The previous data published by Kozár (2009) on WPS males captures during 2007-2008 (Fig. 2) in a ddition to the data collected in this study were used to accurately update the distribution of *P. pentagona* from 2007-2010.

Data were analyzed by using two way ANOVA followed by post hoc Student–Newmann– Keul's multiple-range tests (*a* = 0.05) (CoStat 2004). The collected data of male catches during 2009 and 2010 were statistically compared only with that of Kozár's during 2008 (Kozár 2009) because the numbers of tested stops during 2007 were much lower. Data (mean numbers per trap) for each highway stop (replicates) were ln (x + 1) transformed prior to analysis to normalize the residuals. Bars in the graphs were followed with total number of catches per trap prior to transformation.

Results

The geographical abundances of WPS males and its parasitoids in the tested stops (32 stops) of the five Hungarian highways during the study period (2007-2010) are presented in Figures 2, 3 and 4. The number of WPS males highly varied among the stops and the highways tested. The highest numbers of males captured in pheromone traps were recorded in Csepeli (820), Dunakeszi (16,840), Vecsés (24,200), and Vecsés (16,300) stops of M0 during 2007, 2008, 2009, and 2010, respectively, while the highest number of parasitoid species was recorded in Kisbag stop of M3 during 2009 (28) and 2010 (24) (Fig. 4).

There was no significant difference between the number of males captured between 2009 and 2010 (F= 2.2, df= 1, 63, P >0.05). However, there were significant differences between the number of males captured among the five highways during both years of study (F = 9.28, df = 4,63, P <0.0001). The interaction of "year" by "highway" on the number of catches was not significant (F = 0.82, df = 4,63, P > 0.05) (Fig. 5). The WPS male catches significantly decreased from 2008 to 2010 (F = 3.94, df = 2,85, P < 0.05, Fig. 6).

Eight hymenopterous parasitoids were captured in the WPS pheromone traps during the course of study. These parasitoids were *Thomsonisca amathus* (Walker 1838), *Arrhenophagus* sp. (Encyrtidae: primary parasitoids of WPS), *Encarsia berlesei* (Howard 1906), *Encarsia perniciosi* (Tower 1832), *Encarsia citrina* (Crawford 1891) (Aphelinidae; primary parasitoids of WPS, SJS, and several diaspidid species, respectively), *Coccophagus* sp. (Aphelinidae; primary parasitoid of Coccoidea), *Aphytis* spp. (≈2 species) (Aphelinidae; primary parasitoid of several diaspidid species), and *Signiphora* sp. (Signiphoridae; primary parasitoid of diaspidid species among them WPS). *Arrhenophagus* sp., *E. citrina*, and *E. perniciosi* were recorded but in very low numbers. The spatial distribution of the parasitoid species in the five Hungarian highways is shown in Figure 7. The M0 and M7 highways harbored the highest numbers of parasitoid species. *Coccophagous* sp. was the predominant species in the pheromone traps of WPS. *E. berlesei* which is a main parasitoid of WPS has been captured in low numbers in the traps. The M1 harbored the lowest numbers of all parasitoid species captured. The identity of *P. pentagona* males and *T. amathus* parasitoids was proved by molecular markers, too.

Discussion

The highway studies could supply exact data for the dispersal of an insect pest and its parasitoids. Such data may serve as a basis for forecasting new infestations in countries or parts of countries. Furthermore, changes in actual distribution as compared to the forecast can be evaluated by periodical regional surveying (Kozár and Konstantinova 1981a). These data may also contribute to the development of a 3-4 dimensional European insect thermometer program, which is under development from 2006 (Kozár 2009).

The current study and the previous one by Kozár (2009) revealed that the population of WPS males on highways was much lower compared to the tested stops around the town center (*i.e.*, M0). This may be due to the fact that the diaspidid species prefer areas of mild climate within the temperate and humid zones (Beardsley and Gonzalez 1975). Sheble and Kozár (1996) indicated that the population of WPS in the second generation increased significantly when humidity had been high in the first generation.

The catches of WPS males at newly opened highway stops indicate that the spread potential of WPS is high. This may be associated with the great population of WPS males compared to their females, i.e., male biased sex ratio (Nur 1990, Pedata et al. 1995) or/and the effective spreading of WPS males by vehicles ("transport vector") (Kozár 2009).

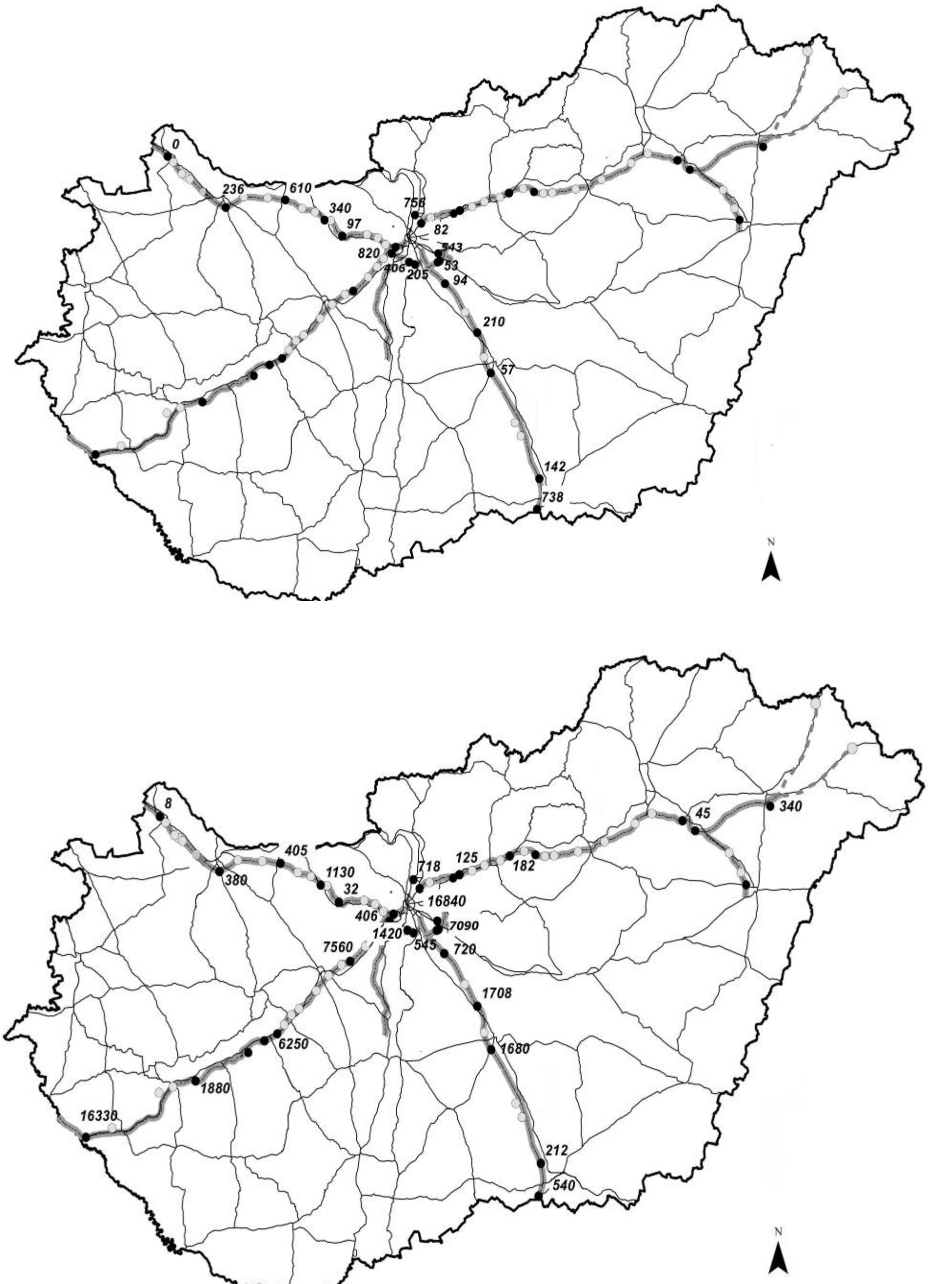

FIG. 2. Geographical maps of males captured per pheromone trap of WPS in the different stops of the main Hungarian highways during 2007 (upper) and 2008 (lower) (Kozár 2009).

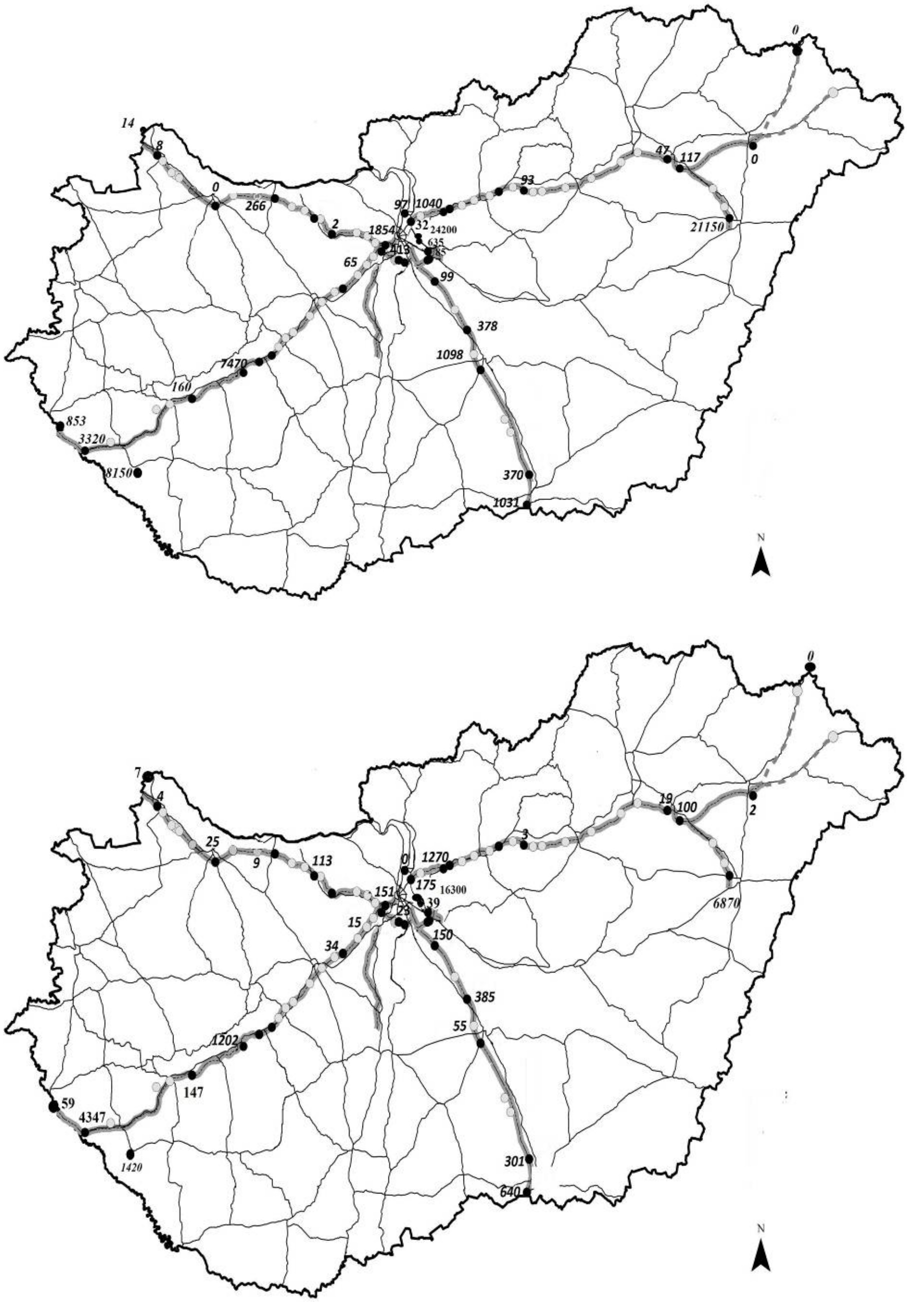

FIG. 3. Geographical maps of males captured per pheromone trap of WPS in the different stops of the main Hungarian highways during 2009 (upper) and 2010 (lower).

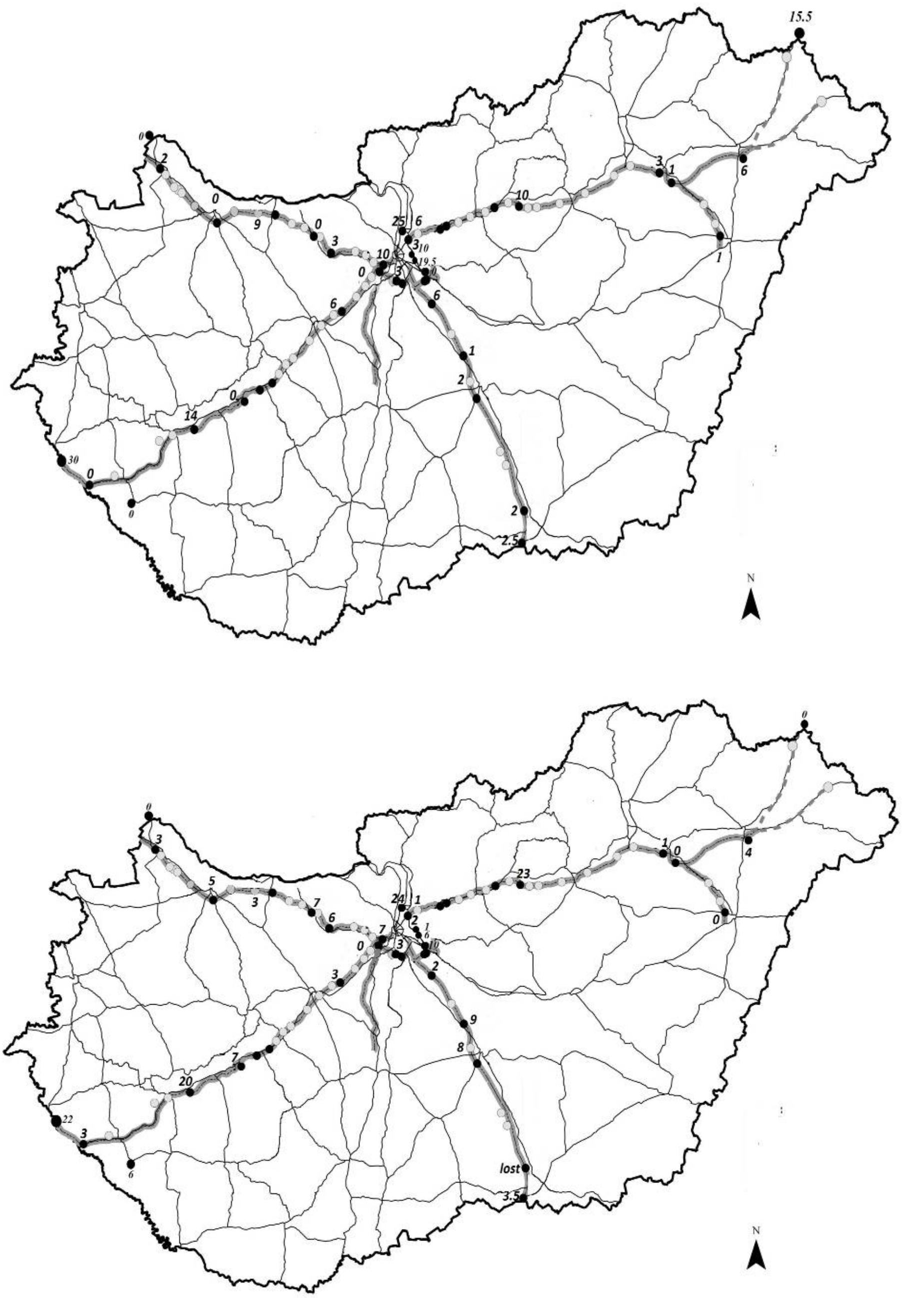

FIG. 4. Geographical maps of total numbers of parasitoid species captured per pheromone trap of WPS in the different stops of the main Hungarian highways during 2009 (upper) and 2010 (lower).

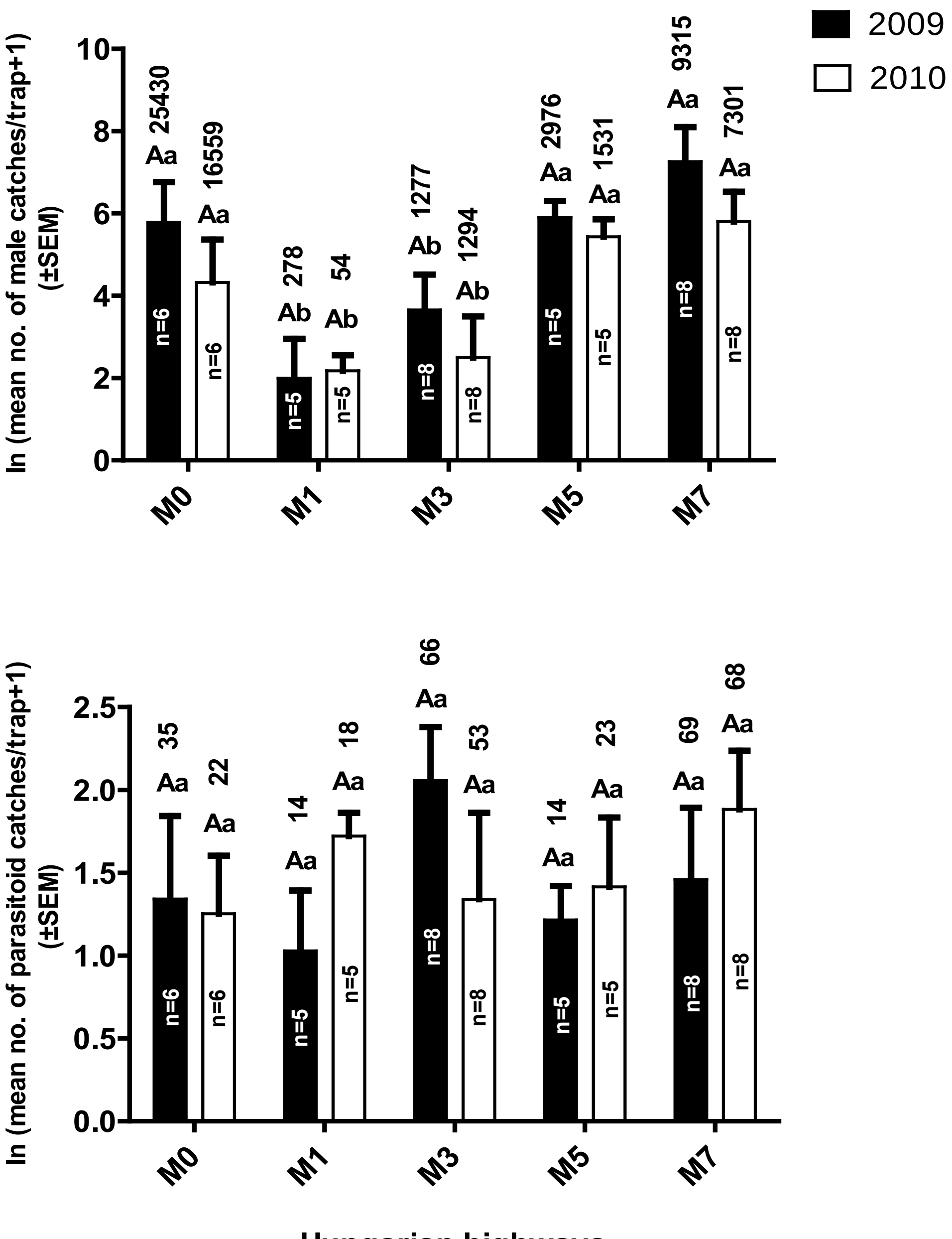

FIG. 5. Mean numbers (ln+1) of *P. pentagona* males (upper) and parasitoids (lower) captured in pheromone traps during the different dates in 2009 and 2010. Bars followed by different capital (between numbers in each highway) and small (among numbers in all highways) letters are significantly different at a=0.05. (Columns are followed by total number of catches per trap within each highway prior to transformation).

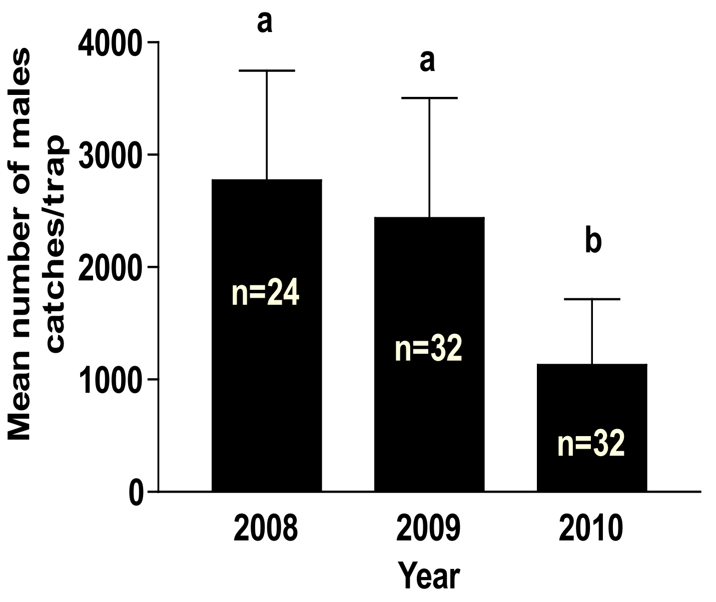

FIG. 6. Mean number of *P. pentagona* males captured in pheromone traps at different stops of Hungarian highways during the different years of the study. Bars followed with the different small letters are significantly different at a=0.05.

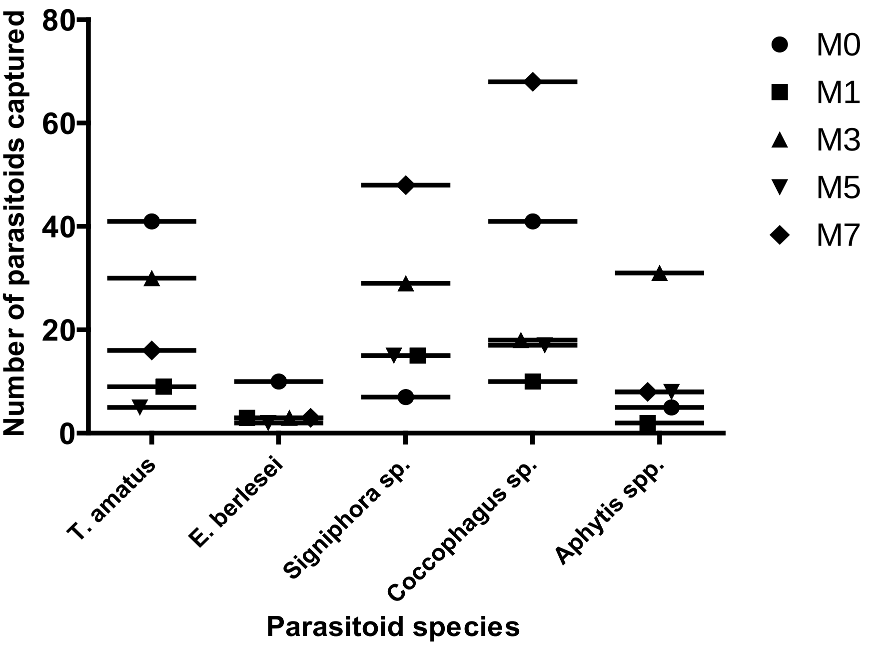

FIG. 7. The spatial distribution of WPS parasitoids captured in pheromone traps at the five Hungarian highways during the years 2009 and 2010.

The significant decrease in the male catches of WPS from 2008 to 2010 indicates a steady decrease of the population of WPS in the last years. The variation in the level of an insect population in long-term depends on biotic or/and abiotic factor associations.

The number of parasitoid species captured was very low (Fig. 5). This decline in parasitoid population may be due to adverse winter conditions. Although the sex pheromone released by WPS females is not known to attract parasitoids (Kozár et al. 1997), the low number of parasitoid records further suggests that WPS pheromone might not have any kairomonal effect and thus no adverse effect of WPS pheromone on beneficial insects are anticipated from its practical use. There were significant differences in the numbers of WPS males captured at the five highways. This result may be due to the variation in the weather conditions and especially the wind (Kozár et al. 2009) or/and the differences in the pest infestation levels among the different areas. The highest numbers of WPS male catches were in M0 followed by M5 and M7. This implies that WPS males may fly from the the northern east (M5) and southern west portions (M7) to accumulate in the humid and warmer sites around the town center (M0), a movement that depends on wind. This pattern of flight activity could be suggested based on the fact that this species originated from the parts of the oriental region (Kosztarab and Kozár 1988), in which the species lives in forests. Thus, the WPS population dispersal in new areas should be monitored strarting from these margins.

The previous studies pointed out that the aphelinid parasitoid *E. berlesei* is the predominant and efficient parasitoid of WPS (*e.g.*, Paloukis et al. 1997, Erler and Tunç 2001); however, its captures in WPS pheromone traps were very low. *Coccophagus* sp. had the highest population among the parasitoids captured in M7. Although *Coccophagus kuwanai* (Silvestri 1927) is a parasitoid attacking nymphs and adults of WPS (Waterhouse and Norris 1987), it is difficult to give evidence that this high attractance is due to a kind of displacement for *E. berlesei* by *Coccophagus* sp. in this highway like that reported in *E. berlesei* by *Encarsia diaspidicola* (Silvestri 1909, Liebregts et al. 1989) or, alternatively, due to high infestation of another coccid species harbored along this highway. The knowldege on the species identity could assist to verify this record.

The continuous monitoring for this serious pest on a large geographical scale using pheromone traps could be useful to determine its dispersal.

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Γεωγραφική μελέτη διασποράς του *Pseudaulacaspis pentagona* και των παρασιτοειδών του σε αυτοκινητόδρομους στην Ουγγαρία με τη χρήση φερομονικών παγίδων και μοριακών δεικτών

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ΠΕΡΙΛΗΨΗ

Πραγματοποιήθηκε μελέτη διασποράς του *Pseudaulacaspis pentagona* (Targioni Tozzetti) (Hemiptera: Diaspididae) και των παρασιτοειδών του σε 32 σημεία κατά μήκος των Ουγγρικών αυτοκινητοδρόμων (M0, M1, M3, M5, και M7) με τη χρήση φερομονικών παγίδων κατά τα έτη 2009 και 2010. Κατά τη μελέτη νέα σημεία συμπεριλήφθηκαν και σε συνδυασμό με προηγούμενα δεδομένα έγινε προσπάθεια κατανόησης της τάσης διασποράς του εντόμου. Ο αριθμός αρσενικών στους αυτοκινητοδρόμους ήταν χαμηλότερος σε σχέση με σημεία κοντά σε πόλεις (M0). Τα δεδομένα συνάδουν με αυτά άλλων μελετών όπου συμπεραίνεται ότι το είδος *P. pentagona* διασπείρεται μέσω οχημάτων κατά μήκος των αυτοκινητοδρόμων (“transport vector”). Η σημαντική μείωση των συλλήψεων αρσενικών από το 2007 έως το 2010 μπορεί να σηματοδοτεί την έναρξη μιας περιόδου χαμηλού πληθυσμού του εντόμου στην Ουγγαρία. Οκτώ διαφορετικά είδη παρασιτοειδών βρέθηκαν στις φερομονικές παγίδες. Το παρασιτοειδές *Coccophagus* sp. ήταν το κυρίαρχο είδος, ωστόσο ενδεχομένως να προέρχεται από άλλο κοκκοειδές. Η ταυτότητα των αρρένων κοκκοειδών καθώς και ενός παρασιτοειδούς επαληθεύτηκε με μοριακούς δείκτες.